510(k) SUBTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:
k070083

B. Purpose for Submission:
This is a new submission

C. Measurand:
Anti-human transglutaminase (h-tTG) and anti-Deamidated Gliadin Peptide (DGP), IgG and IgA Antibodies

D. Type of Test:
Semi-quantitative, ELISA

E. Applicant:
INOVA Diagnostics, Inc.

F. Proprietary and Established Names:
QUANTA Lite™ h-tTG/DGP Screen

G. Regulatory Information:

1. Regulation section:
   21 CFR§866.5660 Multiple autoantibodies immunological test system
   21 CFR§866.5750 Radioallergosorbent (RAST) immunological test system
   Classification:
   Class II
2. Product code:
   MVM autoantibodies, endomysial (tissue transglutaminase)
   MST Antibodies, gliadin
3. Panel:
   Immunology 82

H. Intended Use:

1. Intended use(s):
   The QUANTA Lite™ h-tTG/DGP Screen is an enzyme-linked immunosorbent assay (ELISA) for the semi-quantitative detection of IgA and IgG antibodies to synthetic, deamidated gliadin-derived peptides and human tissue transglutaminase (h-tTG) [endomysium] in human serum. The presence of these antibodies can be used in conjunction with clinical findings and other laboratory tests to aid in the diagnosis of both IgA sufficient and IgA deficient celiac disease.
2. Indications(s) for use:
   Same as intended use.
3. Special conditions for use statement(s):
   For Prescription use only
4. Special instrument requirements:
   Microplate reader capable of measuring OD at 450 nm (or 620 for dual wavelength readings).

I. Device Description:
Each device contains the following: polystyrene microplate strips with breakaway (12-1x8) mirowells coated with h-tTG and synthetic DGP antigen with holder; high positive,

low positive and negative controls (human serum); HRP wash concentrate; HRP sample diluent; HRP IgG and IgA (goat), anti-human conjugate; TMB chromogen and HRP stop solution (0.344M sulfuric acid).

J. Substantial Equivalence Information:

1. Predicate device names(s):
   QUANTA Lite™ Celiac DGP Screen, QUANTA Lite™ h-tTG IgA and the QUANTA Lite™ h-tTG IgG
2. Predicate 510(k) number(s):
   k062708, k011566 and k011570
3. Comparison with predicate:

Similarities				
Items	New Device	Predicate Device	Predicate Device	Predicate Device
	QUANTA Lite™ h-tTG/DGP Screen	QUANTA Lite™ Celiac DGP Screen	QUANTA Lite™ h-tTG IgA	QUANTA Lite™ h-tTG IgG
Intended Use	To aid in the diagnosis of both IgA sufficient and IgA deficient celiac disease	To aid in the diagnosis of both IgA sufficient and IgA deficient celiac disease as well as dermatitis herpetiformis	To aid in the diagnosis of celiac disease and dermatitis herpetiformis.	To aid in the diagnosis of celiac disease and dermatitis herpetiformis.
Technology	ELISA	Same	Same	Same
Assay Format	Semi-quantitative	Same	Same	Same
Platform	96 well microtiter plates	Same	Same	Same
Negative Control	Human serum. Ready to use.	Same	Same	Same
Sample type and dilution	Serum at 1:101	Same	Same	Same
Sample volume	5 µL	Same	Same	Same
Enzyme conjugate	Horseradish peroxidase	Same	Same	Same
Substrate	TMB Chromogen	Same	Same	Same
Incubation times	30-30-30 minutes	Same	Same	Same
OD readings	450 nm (or 620 nm for dual wavelength)	Same	Same	Same
Cut-off	20 units	Same	Same	Same

Differences				
Items	New Device		Predicate Device	Predicate Device
	QUANTA Lite™ h-tTG/DGP Screen	QUANTA Lite™ Celiac DGP Screen	QUANTA Lite™ h-tTG IgA	QUANTA Lite™ h-tTG IgG
Antibody isotype	IgG and IgA for both h-tTG and DGP	IgG and IgA for DGP	IgA for h-tTG	IgG for h-tTG
Antigen	h-tTG and synthetic DGP	Synthetic DGP	h-tTG	h-tTG

K. Standard/Guidance Referenced (if applicable):

None referenced.

L. Test Principle:

Human tissue transglutaminase and purified synthetic gliadin peptides are bound to the wells of a polystyrene microwell plate under conditions that will preserve the antigen in its native state. Pre-diluted controls and diluted patient sera are added to separate wells, allowing any IgA or IgG antibodies present to bind to the immobilized antigen. Unbound sample is washed away and an enzyme labeled anti-human IgA and IgG conjugate is added to each well. A second incubation allows the enzyme labeled anti-human IgA and IgG to bind to any patient antibodies, which have become attached to the microwells. After washing away any unbound enzyme labeled anti-human IgA and IgG, the remaining enzyme activity is measured by adding a chromogenic substrate and measuring the intensity of the color that develops using a spectrophotometer and comparing the color intensity that develops in the patient wells with the color in the control wells.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. *Precision/Reproducibility:*

The intra-assay precision was determined using twelve serum samples, with h-tTG/DGP concentration levels ranging from 6.8 to 113.3 units with four positive samples, two low positives just above the cut-off of 25 units, two high negatives and four negatives. These twelve samples were tested five times. The positive samples had ≤1.7% C.V. and the negative samples had ≤12.4% C.V. as shown below.

Intra-assay Performance of QUANTA Lite™ h-tTG/DGP Screen ELISA

	1	2	3	4	5	6	7	8	9	10	11	12
Mean units	113.3	62.9	41.3	35.7	22.1	21.6	18.7	19.0	7.9	6.8	12.2	7.0
SD	1.3	1.1	0.7	0.6	0.4	0.3	0.2	0.9	1.0	0.5	0.6	0.3
CV%	1.2	1.7	1.7	1.7	1.9	1.2	1.2	4.5	12.4	7.7	4.6	4.6

The inter-assay precision was determined using twelve serum samples assayed in duplicate, twice daily for three days. This study was performed by single operator at INOVA Diagnostics using one kit lot. The positive samples had ≤9.5% C.V. and the negative sample had ≤21.3% C.V. (see table below).

Inter-assay Performance for QUANTA Lite™ h-tTG/DGP Screen ELISA

	A	B	C	D	E	F	G	H	I	J	K	L
Mean units	66.6	117.1	46.4	22.4	36.8	22.9	16.1	17.1	7.2	6.6	6.6	9.7
SD	2.1	3.6	2.4	1.2	3.3	2.2	1.1	1.5	0.3	0.7	1.4	1.8
CV %	3.2	3.0	5.2	5.3	9.0	9.5	6.9	8.7	4.4	11.2	21.3	18.8

Lot to lot variation was performed at INOVA Diagnostics by three different operators using three different kit lots on three different days. A summary of the results of the study is shown below.

Sample	Lot 1	Lot 2	Lot 3	Mean Units	Std Dev	%CV
High Positive Control	88.23	77.39	98.90	88.17	10.76	12.20
Positive 1	53.22	57.92	58.94	56.69	3.05	5.38
Positive 2	104.68	118.56	110.69	111.31	6.96	6.25
Positive 3	44.16	43.85	44.83	44.28	0.50	1.13
Positive 4	27.31	21.24	29.48	26.01	4.27	16.42
Positive 5	30.63	36.85	37.25	34.91	3.71	10.63
Positive 6	22.23	23.23	21.74	22.40	0.76	3.39
Positive 7	40.39	47.32	46.74	44.82	3.84	8.58
Positive 8	114.13	121.18	114.36	116.56	4.01	3.44
Reagent Blank	1.66	1.66	1.96	1.76	0.17	9.84
Negative Control	1.71	1.54	1.96	1.74	0.21	12.17
Negative 1	6.64	5.64	7.43	6.57	0.90	13.65
Negative 2	6.44	6.61	8.63	7.23	1.22	16.86
Negative 3	8.55	8.94	9.19	8.89	0.32	3.63
Negative 4	8.55	11.90	12.45	10.97	2.11	19.25

b. *Linearity/assay reportable range:*
Not applicable.

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*
There is no reference standard for anti-tTG and anti-gliadin antibodies. The positive and negative controls are prepared in house and arbitrary units are assigned during the development process.

d. *Detection limit:*
Not applicable.

e. *Analytical specificity:*
Cross-reactivity
To assess potential cross reactivity problems with other autoantibodies, a variety of high titer autoantibody positive samples were run on the QUANTA Lite™ h-tTG/DGP Screen kit. In all, 83 samples were run. Many of the samples were the high positive controls from other INOVA QUANTA Lite™ autoantibody test kits. The specificities included Actin (4), Chromatin (7), dsDNA (4), M2 (4), MPO (5), Centromere (7), CCP3 (5), GBM (9), Ribo P (4), RF IgG (4), SS-B (4), RNP (4), Scl-70 (4), Jo-1 (4), Sm (4), SS-A (6) and TPO (4). All of the samples were negative on the QUANTA Lite™ h-tTG/DGP Screen with a mean value of 4.51 units which is 4.4 SD below the cut-off of 20

units.

Interfering substances

Interference by endogenous substances: No data provided. The package insert states that microbial contamination, heat-treated, or specimens containing visible particulate should not be used. Grossly hemolyzed or lipemic serum or specimens should be avoided in this assay.

f. *Assay cut-off:*

INOVA uses a cutoff between positive and negative of 20 units for the purpose of continuity with the predicate devices.

2. Comparison studies:

a. *Method comparison with predicate:*

Testing was preformed on 99 samples from three celiac disease reference labs, plus 496 normals. Samples clinically defined as either celiac patients, celiac patients that are IgA deficient, celiac patients on a gluten-free diet, first degree relative of a celiac patient or IgA deficient controls were included in this study.

N= 595		Celiac DGP Screen and/or h-tTG	
		Positive	Negative
h-tTG/DGP Screen	Positive	48	4*
	Negative	7**	536

*Of the 4 samples found to be h-tTG/DGP Screen positive, yet negative on both the Celiac DGP and h-tTG kits, 3 were from the normal subjects. The fourth patient was a celiac patient on a gluten free diet, 22.1 units.

**Of the seven samples found negative by the h-tTG/DGP Screen kit yet positive by either the Celiac DGP or h-tTG kits, 5 were from normal subjects. Of the remaining, one sample was from a celiac patient on a gluten free diet with 21.5 units on h-tTG IgA. The other sample was a first degree relative, 20.2 units on Celiac DGP Screen.

Positive percent agreement: 48/55 (87.3%) (95% CI: 75.5% to 94.7%)
Negative percent agreement: 536/540 (99.3%) (95% CI: 98.1% to 99.8%)
Overall agreement: 584/595 (98.2%) (95% CI: 96.7% to 99.1%)

b. *Matrix comparison:*

Not applicable.

3. Clinical studies:

a. *Clinical Sensitivity and Specificity:*

Samples clinically defined as either celiac patients untreated (139 are celiac pediatric patients), celiac patients that are IgA deficient, latent celiac patients, refractory celiacs, celiac patients on a gluten-free diet, first degree relatives of a celiac patient, IgA deficient controls or Non-Celiac Disease Patients were tested in both internal and external clinical studies using the QUANTA Lite™ h-tTG/DGP Screen. A summary of the clinically defined samples and normal range samples is provided below.

Patient Group	#	# positive	% positive
Celiacs untreated (pediatric)	185 (139)	183 (139)	99% (100%)
Celiac IgA Deficient	5	5	100%
Celiac, latent	11	10*	91%

Patient Group	#	# positive	% positive
Celaic, refractory	9	8**	89%
Celiacs on Gluten-Free Diet	120	34	28%
1st degree relatives	18	2	11%
Disease Controls	76	8	10.5%
Normals	914	20***	2.2%

*8 of the 11 latent celiacs were tTG IgA positive and 9 were EMA positive
**None of these 9 refractory celiacs were EMA positive and only 2 (22%) were tTG positive
***2 of the 20 positives were found to be positive on individual tTG and DGP ELISA assays and also positive for tTG by fluid phase RIA.

Sensitivity – Overall sensitivity for celiac disease is calculated by grouping the results for all the celiac patients except those that are already on a gluten free diet. The total number of celiacs is 210 of which 207 or 98.6% are positive.

Specificity – Specificity can be calculated by grouping the 914 normals, the 76 disease controls and the 18 first-degree relatives. This group totals 1008 samples. Of these, 30 samples were positive for a specificity of 97%.

h-tTG/DGP Screen ELISA Performance with Celiac Diagnosis*

		Diagnosis		
		Positives (Celiacs untreated, pediatric, IgA deficient, latent and refractory)	Negative (1st degree relatives, Disease Controls and Healthy Controls)	Totals
QUANTA LITE™ h-tTG/DGP Screen	Positive	207	30	237
	Negative	3	978	981
	Total	210	1008	1218

*Does not include the 120 celiac patients on a gluten free diet.

Sensitivity: 98.6% (207/210) (95% CI: 95.9-99.7%)
Specificity: 97.0% (978/1008) (95% CI: 95.8-98.0%)

b. *Other clinical supportive data (when a. is not applicable):*
Not applicable.

4. Clinical cut-off:
Same as assay cut-off.
5. Expected values/Reference range:
Four hundred and ninety-six random asymptomatic, healthy individuals residing in the USA were tested for antibodies to h-tTG/DGP. Of these, 299 subjects were known to range in age from 14y to 78y and they included 150 males and 149 females. Seven samples (1.4%) were above the 20-unit cutoff. Five of these samples had anti-tTG/DGP values of 20.2, 22.1, 23.4, 29.2 and 36.5 units. The other two positive samples were 46.1 and 39.0 units and believed to be from true celiacs due to the fact that the h-tTG IgA results were 57.2 and 72.4 units. The mean value of the 496

samples was 6.2units. The standard deviation of the samples was 3.8 units. The mean value is three standard deviations below the 20-unit cutoff.

The values shown below are suggested values only. Each laboratory should establish its own normal range.

	Units
Negative	<20
Positive	≥20

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:

The submitted information in this premarket notification is compete and supports a substantial equivalence decision.